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BIOAEROSOL EXPOSURE TO FILTERING FACEPIECE RESPIRATORS IN A CLINICAL ENVIRONMENT

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14. ABSTRACT

Nosocomial infections affect 5 -10% of hospital admissions and pose a significant threat to healthcare workers (HCWs). Evolving antibiotic resistance of virulent and commensal strains is leading to more severe hospital-acquired infections. This study evaluated bioaerosol contamination of filtering facepiece respirators (FFRs) worn by hospital staff. Such data are needed to understand respiratory hazards for HCWs and the amount of contamination found on FFRs. Hospital environmental staff wore 3M1860 or 3M1870 FFRs during cleaning of discharged patient rooms. Coupons were cut from the FFRs, then the external and filtering layers were extracted. Extracts were plated on permissive media and all colonies were counted. 1.6% of isolates were characterized by biochemical and antimicrobial resistance testing using vancomycin and oxacillin. Average loading of microbes ranged from 6.2 x 10^2 -4.8 x 10^3 colony-forming units per mask. ~97% of the contamination was found on the external layer. Most of the isolates recovered were coagulase-negative, Gram-positive staphylococci and *Micrococcus* spp . 73% of the Gram-positive and 67% of the Gram-negative isolates were resistant to oxacillin. Vancomycin resistance was lower --9.2% and 36.7%, respectively. Our data confirm the presence of antibiotic-resistant microorganisms in hospital air, and the attendant threat to hospital occupants. An estimate is provided for mask bioburden loading that can be used to refine FFR reuse strategies.

15. SUBJECT TERMS

Bioaerosol, nosocomial, oxacillin, pathogen, resistant, respirator, vancomycin

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Bioaerosol Exposure to Filtering Facepiece Respirators in a Clinical Environment

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SUMMARY
Background: Nosocomial infections affect 5–10% of hospital admissions and pose a significant threat to healthcare workers (HCWs). Antibiotic resistance of virulent and commensal strains is increasing, leading to more severe hospital-acquired infections.
Aims: Our aim was to evaluate aerosol contamination of filtering facepiece respirators (FFRs) worn by hospital staff. Such data are needed to understand respiratory hazards for HCWs and the amount of contamination found on FFRs.
Methods: Hospital environmental staff wore 3M1860 or 3M1870 FFRs during cleaning of discharged patient rooms. Coupons were cut from the FFRs, then the external and filtering layers were extracted. Extracts were plated on permissive media and all colonies were counted. 1.6% of isolates were characterized by biochemical and antimicrobial resistance testing using vancomycin and oxacillin.
Findings: The average loading of microbes ranged from 6.2×10^2–4.8×10^3 colony-forming units per mask. ~97% of the contamination was found on the external layer. Most of the isolates recovered were coagulase-negative, Gram-positive staphylococci and *Micrococcus* spp . 73% of the Gram-positive and 67% of the Gram-negative isolates were resistant to oxacillin. Vancomycin resistance was lower—9.2% and 36.7%, respectively.
Conclusion: Our data confirm the presence of antibiotic-resistant microorganisms in hospital air, and the attendant threat to hospital occupants. We provide an estimate for mask bioburden loading that can be used to refine FFR reuse strategies.

KEY WORDS:
Respirator, bioaerosol, nosocomial, resistant, pathogen, oxacillin, vancomycin,

INTRODUCTION

The evolution of antimicrobial resistance in microorganisms and the continual emergence of infectious diseases have made the hospital an increasingly hazardous environment. Hospital-acquired infections (HAIs) affect 5–10% of all hospitalizations in the U.S. annually,[1] largely caused by the development of antibiotic resistance in pathogenic and commensal microorganisms.[2,3] The environmental stability of these microbes allows them to remain viable on surfaces for days, whence they may transmit to hospital occupants.[4,5] Viable pathogens have previously been found in hospital air in concentrations that may warrant concern.[6,7,8] Due to their route of exposure, airborne microorganisms require a much different risk assessment than those found on surfaces.

Infection control practices are implemented to suppress HAIs, but limiting airborne transmission provides a difficult challenge. Traditional approaches to reduce surface contamination are only marginally effective, and thus airborne microbes derived from surface reaerosolization persist. Greene, *et al*., measured airborne concentrations of bacteria as high as 7×10^3 colony-forming units (CFU) /m^3, which fluctuated with traffic patterns.[6] Airborne *Staphylococcus aureus* was also correlated with airborne skin cells that provide a constant supply of surface and airborne microorganisms.[9] The Occupational Safety and Health Administration (OSHA) mandates that hospitals develop and implement a respiratory protection (RP) plan for workplace hazards that involve respiratory threats.[10] However, RP standards are primarily enforced when exposure to aerosolized pathogens is a concern due to high risk patients or a pandemic.[11] Risks posed by

aerosolized environmental contaminants have not elicited enough concern to invoke the routine use of RP.

The National Institute for Occupational Safety and Health (NIOSH)-approved N95 filtering facepiece respirator (FFR) is the recommended device for protecting workers from respiratory pathogens. FFRs garnered significant attention due to an anticipated shortage caused by pandemic influenza. A large body of work is reported on FFR decontamination, cleaning and reuse (FDCR), but the data are difficult to apply to a real-world scenario without data on FFR bioburden during use.[12,13,14] This study evaluates the bacterial bioburden found on FFRs worn by environmental staff in the hospital setting. The data from this study fill gaps concerning FDCR and also provide data on exposure of healthcare workers (HCWs) to aerosolized microorganisms.

MATERIALS AND METHODS

Twenty-five 3M1860 and twenty-seven 3M1870 FFRs, NIOSH-approved surgical N95s commonly worn in hospitals, were used for this study. Prior to use, they were treated with ultraviolet germicidal irradiation at 20 mW/cm^2.

Bay Medical Center/Sacred Heart Health System (BMC) of Panama City, FL, was the test site. Volunteers from the environmental staff wore FFRs during their duties cleaning discharged patient rooms. The Internal Research Committee at BMC approved the study design. The staff was instructed how to properly don and doff FFRs to avoid contact contamination. Participants wore two pairs of gloves before donning the respirator. No contact with patients or other personnel occurred during the cleaning procedure. Average wear time was 20 minutes. Immediately after cleaning patient rooms, participants removed their outer gloves, doffed the FFR and placed it in a sterile bag containing sterile cotton plugs wetted with 5 mL of water. The bag was sealed and placed on ice for ~18 hours.

Three 38-mm diameter circular coupons were removed from the front section of each FFR using a sterile punch. Coupon layers were separated with forceps; only the filtering (middle) layer and external layer were evaluated. Like-coupon types were co-extracted in 50-mL tubes containing 10 mL of extraction buffer for five minutes using a vortex mixer.[12]. A 5-mL aliquot of extraction fluid was filtered through a sterile (0.45 µm × 47 mm diameter) GN-6 Metricel® MCE filter (Pall Corporation, Ann Arbor, MI). The filter was incubated at 37 °C for 48 hours on trypticase soy agar (TSA). Following incubation, colonies were counted and the microorganism concentration was determined using Equation 1.

$$V = 2n \div 34 \text{ cm}^2 \qquad \textbf{(Equation 1)}$$

V = Viable bacteria extracted per cm^2 of FFR
n = Number of bacterial colonies on TSA plate.

A directed approach based on colony morphology was used to select isolates for characterization. Colonies were streaked for isolation on TSA plates and incubated overnight at 37 °C. Purified isolates were characterized by Gram staining. Gram-negative isolates were identified using the 20E API® system (bioMérieux Clinical Diagnostics, Marcy l'Etoile, France); Gram-positive isolates were identified using the API® Staph test system according to the manufacturer's

instructions. Isolates were tested for resistance to vancomycin and oxacillin using standard protocols.[15]

RESULTS

The mean concentration of viable bacteria found on the external and filtering layer of the 3M1860 and 3M1870 FFRs was 24.15 and 0.6 CFU/cm^2; and 3.33 and 0.1 CFU/cm^2, respectively (Figure 1). An unpaired two-tailed *t*-test demonstrated the comparison of the external and internal layers between both FFRs was statistically significant ($P < .05$). The data were highly variable and the standard deviations exceeded the mean values in all samples. External layers of the 3M1860 and 3M1870 FFRs captured 97.5% and 96.9% of contamination, respectively.

The recovered bioburden from the worn FFRs produced ~1.2×10^4 isolates, of which 196 were selected for further analysis; 147 Gram-positive and 49 Gram-negative. From 138 of these, API analysis yielded identification of 23 species at confidence levels of 30.0–99.9%; the remaining 58 were not identified (Table 1).

Antimicrobial resistance to oxacillin was found in 73.1% of Gram-positive and 67.3% of Gram-negative isolates (Table 2). Vancomycin resistance was lower—9.2% and 36.7%, respectively. More Gram-negative (32.65%) than Gram-positive (8.5%) bacteria showed resistance to both antibiotics.

DISCUSSION

This study provides data that quantify bacterial contamination of FFRs worn in hospital settings and provides a measure of the potential risk of inhalable microorganisms. Previous studies measuring airborne concentrations of microorganism in hospitals used methods that don't mimic human respiration and thus are limited in their interpretations.[6,9,16] We acknowledge that this study also has limitations: Only $n = 25$ and 27 of two FFR models were used; isolation media biased sampling; only 1.6% of the isolates were characterized; no patient interaction occurred; and significant differences between FFR model data were observed due to FFRs worn by different participants at different locations in the hospital. The strength of the study is the use of humans as "bio-collectors."

Our data demonstrated that an FFR worn for 20 minutes acquired 3–24 CFU/cm^2 of external surface contamination. Based on ~200 cm^2 total surface area of a typical FFR, total loading would be 6.0×10^2–4.8×10^3 CFUs, assuming even distribution of the microbes on the FFR. Loading of the filtering layer was 0.1–0.6 CFU/cm^2 (20~120 CFU per FFR, twice the loading found on surgical masks worn by dentists during routine procedures with patients).[17] Extended wear times would likely have yielded more contamination.

These data are important for development of FDCR strategies because no data existed documenting contamination found on FFRs during use. In laboratory studies, *S. aureus* applied to FFRs via aerosol deposition was reduced by four logs using cleaning wipes containing bleach or benzalkonium chloride.[18] The mean bacterial accumulation on FFRs in this study was 2.7×10^3 CFU during a 20-minute wear time. Wear times could extend to eight hours without exceeding the four-log reduction capability of wipes. It is unclear that higher concentrations of microorganisms would have been acquired during patient contact, but the question merits further study.

It is accepted that the filtering element of the FFR is responsible for particle capture, thus it was surprising that < 4% of the isolates were found on this layer. This indicates that the mean particle size of the aerosols was large and/or that the FFR was contaminated by contact. A previous study found that 78% of the particles in hospital ambient air were >2 µm and the concentration of particles increased (0–7.0 $x10^3$ CFU/m^3) with people movement.[6] Noble and Davies also showed a positive correlation between airborne *S. aureus* and skin cells and defined the average particle size as 13 µm.[9] These data support that contamination of FFRs may occur through the aerosol route and demonstrate why loading occurred on the external layer. Contamination of the mask could have also occurred through touching, but precautions were taken to limit contact. Microbial contamination found on hospital uniforms demonstrated a similar contamination per unit area as observed in this study.[19] Contamination of the uniforms likely occurred via contact transmission, but aerosol contamination may have also contributed to overall bioburden.

Characterization of isolates was performed on 1.6% of the isolates due to logistics. The approach produced a bias, but it is unclear to what degree. API tests (Table 1) identified 70.4% of the isolates; 29.6% fell below the 30% threshold. We identified 26 species, primarily coagulase-negative staphylococci (CNS) and *Micrococcus* spp. *Acinetobacter baumanii/calcoaceticus* and *S. aureus* were identified, but in low numbers (Table 1). It is noteworthy that no vancomycin-resistant enterococci (VRE) were identified. Our methods may have biased against their isolation, or they may have occurred in the population not identified by API analysis. CNS, while not as virulent as other agents, are known to cause bloodstream and catheter infections.[5] CNS and *Micrococcus* spp. were also the most prevalent organisms found in multiple hospital surveys, and thus it was no surprise to find them in our study.[20,21,22] Their residency on the skin suggests that contact transfer may have been responsible for deposition, but these same microbes would have the potential for reaerosolization from surfaces and thus deposition by aerosol transmission is plausible.[9] API was unable to identify the majority of Gram-negative isolates, so it is unclear what risks they may pose and their origin.

A high percentage of isolates were resistant to vancomycin and/or oxacillin (Table 2), which is not surprising given the amount of oxacillin-resistant *S. aureus* and VRE found in hospitals. Of only three *S. aureus* isolates identified, two were resistant to oxacillin; 141 additional isolates of other species showed oxacillin resistance as well. Fewer isolates (30) displayed vancomycin resistance, but none were identified as enterococci. The resistance profile of CNS has increased, escalating rates of human infections.[5] Resistant CNS causes many concerns due to horizontal gene transfer, which may allow them to gain virulence factors, or they may serve a reservoir to pass antibiotic-resistant genes to more-virulent strains.[23] A better understanding of the role of antibiotic-resistant CNS is needed to understand their role in HAIs.

Significance of these data to hospital occupants is difficult to quantify. Clearly, a resistant population of microbial isolates exists, and it is known that these microorganisms can be airborne, providing potential for exposure by inhalation.[16] It is not known that HCWs in the presence of patients would have been exposed to similar size aerosols or microorganisms. We also don't know that our data are representative of all hospitals or even other locations within hospitals. There does not appear to be an acute health problem in hospitals due to breathing contaminated air but long-term exposure to antibiotic-resistant pathogens is likely occurring. More work is needed to properly quantify the threat to HCWs.

The use of respiratory protection comes with burdens, financial and physical, that must be weighed against risk factors that evolve as antibiotic resistance of pathogens and commensal microorganisms increases. Our data suggest that hospital occupants are exposed to large particles that could be effectively removed by devices with lower filtration efficiencies than the N95. There is currently a great deal of interest in designing an FFR specifically for HCW, but lowering the filtration efficiency is not being considered.[24] To understand the threat posed to HCW, more data are needed that can be used to develop appropriate requirements for PPE. The sampling strategy used in this study provides the most relevant data for inhalation threats posed to HCW. More-extensive studies in the presence of patients, in isolation wards, and evaluating disease-specific agents are needed to help in that process.

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Table 1. API Match of Isolates Recovered From 3M 1860 and 3M 1870 FFRs

Gram Stain	API Match	Confidence of Match							Total
		100-90 %	89-80 %	79-70 %	69-60 %	59-50 %	49-40 %	39-30 %	
Gram Negative	*Acinetobacter baumannii/calcoaceticu*	1							**1**
	Ochrobactrum anthropi	1			2				**3**
	Pseudomonas fluorescens/putida		2						**2**
	Rahnella aquatilis		1						**1**
	Stenotrophomonas maltophilia				1				**1**
	unacceptable profile	----------							**41**
Gram Positive	*Kocuria kristinae*	1		2					**3**
	Kocuria varians/rosea	1	2				1		**4**
	Micrococcus spp	33	1		1				**35**
	Staphylococcus aureus	1			1	1			**3**
	Staphylococcus auricularis				1	1			**2**
	Staphylococcus capitis		1	1	2				**4**
	Staphylococcus caprae	1							**1**
	Staphylococcus chromogenes		1				2	1	**4**
	Staphylococcus cohnii ssp cohnii	1						1	**2**
	Staphylococcus epidermidis	14	6	6		2	2		**30**
	Staphylococcus haemolyticus			1		1	7		**9**
	Staphylococcus hominis	5		2	4	2	4	1	**18**
	Staphylococcus lentus	2							**2**
	Staphylococcus saprophyticus		1		2	1			**4**
	Staphylococcus schleiferi							1	**1**
	Staphylococcus sciuri			3			1	1	**5**
	Staphylococcus warneri		1						**1**
	Staphylococcus xylosus	2							**2**
	unacceptable profile	----------							**17**

Table 2. Antibiotic Resistance of Isolates Recovered From 3M 1860 and 3M 1870 FFRs

Isolates	API Identification	Oxacillin Resistant	Vancomycin Resistant	Oxacillin and Vancomycin Resistant
Gram Negative	*Acinetobacter baumannii/calcoaceticus*	1/1	1/1	1/1
	Ochrobactrum anthropi	3/3	1/3	1/3
	Pseudomonas	2/2	2/2	2/2
	Rahnella aquatilis	1/1	1/1	1/1
	Stenotrophomonas maltophilia	0/1	0/1	0/1
	Not Identified	26/41	13/41	11/41
	Total	**33/49 (67.3%)**	**18/49 (36.7%)**	**16/49 (32.65%)**
Gram Positive	*Kocuria kristinae*	2/3	0/3	0/3
	Kocuria varians/rosea	2/4	1/4	1/4
	Micrococcus spp.	33/35	5/35	5/35
	Staphylococcus aureus	2/3	0/3	0/3
	Staphylococcus auricularis	2/2	0/2	0/2
	Staphylococcus capitis	2/4	0/4	0/4
	Staphylococcus caprae	1/1	0/1	0/1
	Staphylococcus chromogenes	2/4	0/4	0/4
	Staphylococcus cohnii	1/2	0/2	0/2
	Staphylococcus epidermidis	19/30	0/30	0/30
	Staphylococcus haemolyticus	8/9	1/9	1/9
	Staphylococcus hominis	11/18	0/18	0/18
	Staphylococcus lentus	2/2	1/2	1/2
	Staphylococcus saprophyticus	3/4	0/4	0/4
	Staphylococcus schleiferi	0/1	0/1	0/1
	Staphylococcus sciuri	3/5	0/5	0/5
	Staphylococcus warneri	1/1	0/1	0/1
	Staphylococcus xylosus	2/2	0/2	0/2
	Not Identified	12/17	4/17	4/17
	Total	**108/147 (73.5%)**	**12/147 (8.2%)**	**11/147 (7.5%)**

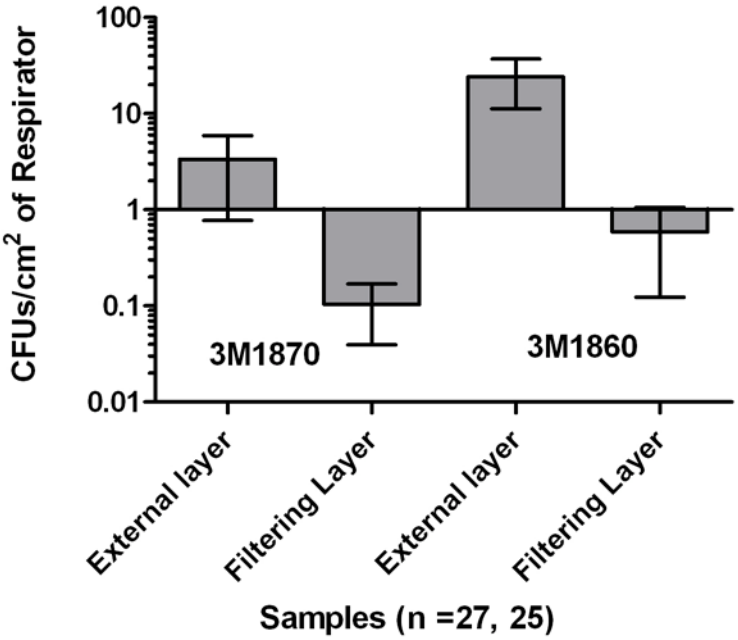

Figure 1. Isolates Recovered From 3M 1860 and 3M 1870 FFRs